

Amendment to the Claims

This listing of Claims will replace all prior versions, and listings, of claims in the application.

Listing of Claims

1. (Currently Amended) A method of crystallization, comprising the steps of:

- a. introducing a first carrier-fluid into a first channel of a substrate;
- b. introducing into a plug-forming region of the first channel:
 - 1) a first plug-fluid comprising a precipitant;
 - 2) a second plug-fluid comprising water; and
 - 3) a third plug-fluid comprising a crystallization target;

where each of the first, second and third plug-fluids comprises a solvent;

where pressure is applied when introducing each of the first, second and third plug-fluids, thereby resulting in the formation of a plurality of plugs in the first channel, at least one of the plugs comprising the first, second and third plug-fluids; and

where the crystallization target forms a crystal in the at least one of the plugs.

2. (Currently Amended) The method of claim 1, further also comprising the steps of:

- a. introducing a second carrier-fluid into a second channel of the substrate;
- b. introducing into a plug-forming region of the second channel:

- 1) a ~~first~~ fourth plug-fluid comprising a precipitant;
- 2) a ~~second~~ fifth plug-fluid comprising water; and
- 3) a ~~third~~ sixth plug-fluid comprising a crystallization target;

where each of the fourth, fifth and sixth plug-fluids comprises a solvent;

where pressure is applied to the second channel when introducing each of the ~~first, second and third~~ fourth, fifth and sixth plug-fluids, thereby resulting in the formation of a plurality of plugs comprising the fourth, fifth and sixth plug-fluids in the second channel;

where the first and second channels merge into a common downstream channel;

where at least one plug flowing from the first channel merges with a plug flowing from the second channel upon entry into the downstream channel.

3. (Currently Amended) A method of crystallization, comprising the steps of:

a. introducing a first carrier-fluid into a first channel of a substrate;
b. introducing one or more first plug-fluids into a first plug-forming region of the first channel to form a plug of a first plug type;

where a plug of the first plug type comprises a precipitant and a crystallization target;

c. introducing one or more second plug-fluids into either one of:
1) the first plug-forming region;
2) a second plug-forming region within the first channel; or
3) a second plug-forming region within a second channel;

where each of the plug-fluids comprises a solvent;

where the one or more second plug-fluids form a plug of a second plug type at either one of the first, second and third plug forming regions the first plug-forming region, the second plug-forming region within the first channel, or the second plug-forming region within a second channel;

where a plug at least one of the plugs of the second plug type comprises a precipitant;

where pressure is applied to the first and second channels when introducing the one or more first plug fluids and the one or more second plug fluids;

where at least one or more plugs of the first plug type alternate in sequence with one or more plugs of the second plug type; and

where the crystallization target forms a crystal in at least one of the plugs at least one or more plugs of the first plug type and the one or more plugs of the second plug type.

4. (Original) The method of claim 1 or 3, where the crystallization target is a member of the group consisting of protein, peptide, polynucleotide, oligonucleotide, subcellular organelle, subcellular protein complex, drug, small molecule/biological macromolecule complex, virus, colloidal particle, nanoparticle and combinations thereof.

5. (Original) The method of claim 1 or 3, where the crystallization target is a protein.

6. (Currently Amended) The method of claim 1 or 3, where there is transfer of a solvent solvent transfer from one plug into another plug.

7. (Original) The method of claim 6, where the solvent is water.

8. (Canceled)

9. (Original) The method of claim 3, where the one or more second plug-fluids is introduced into the first plug-forming region.

10. (Original) The method of claim 3, where the one or more second plug-fluids is introduced into the second plug-forming region.

11. (Currently Amended) The method of claim 3, where the one or more second plug-fluids is introduced into the second channel within the ~~third~~ second plug-forming region.

12. (Currently Amended) The method of ~~claim 3~~ claim 11, where the first and second channels merge into a common downstream channel, such that one or more plugs flowing from the first channel alternate with one or more plugs of the second plug type flowing from the second channel upon entry into the downstream channel.

13. (Currently Amended) The method of claim 9, where a second carrier-fluid is introduced into the first plug-forming region of the first channel so that it separates the one or more first plug-fluids from the one or more second plug-fluids prior to formation of ~~the~~ any plug at the first plug-forming region.

14. (Original) The method of claim 13, where the second carrier-fluid is introduced through an inlet positioned between a first set of one or more inlets used for introducing one or more of the first plug-fluids and a second set of one or more inlets used for introducing one or more of the second plug-fluids.

15. (Currently Amended) The method of claim 1 or 3, where each of the carrier-fluids and plug-fluids is introduced into the substrate according to a ~~given~~

respective flow rate under pressure, either directly into a channel or through an inlet feeding into a channel.

16. (Canceled)

17. (Original) The method of claim 15, where the pressure is halted after one or more plugs are formed.

18. (Canceled)

19. (Currently Amended) The method of claim 15, where at least one of the respective flow rates is varied relative to one another of the respective flow rates.

20. (Canceled)

21. (Currently Amended) The method of claim 15, where at least one of the plug-fluids is introduced into a respective carrier-fluid at a variable respective flow rate.

22. (Currently Amended) The method of claim 15, where the respective flow rates are varied resulting in a plurality of plugs exhibiting a concentration gradient among the plurality of plugs with regard to one or more of the plug-fluids.

23. (Original) The method of claim 1 or 3, where mixing of the plug-fluids within the plugs is increased by adding a turn to a channel.

24. (Currently Amended) The method of claim 3, where each of the carrier-fluids and plug-fluids is introduced into the substrate according to a given respective flow rate under pressure, either directly into a channel or through an inlet feeding into a channel; and where one or more of the respective flow rates are varied so that the concentration of a first component in at least one of the plugs of one plug-type

provides a correlative and quantitative measure of a second component in an adjacent plug.

25. (Original) The method of claim 24, where the first component is a marker.

26. (Original) The method of claim 1 or 3, where at least one of the plug-fluids comprises a salt.

27. (Original) The method of claim 3, where the osmotic pressure in the plug of the first plug type at the first plug-forming region is lower than the osmotic pressure in the plug of the second plug type at the plug-forming region where the plug of the second type is formed.

28. (Original) The method of claim 3, where the osmotic pressure in the plug of the first type at the first plug-forming region is at least 2 times lower than the osmotic pressure in the plug of the second plug type at the plug-forming region where the plug of the second type is formed.

29. (Original) The method of claim 3, where the osmotic pressure in the plug of the first type at the first plug-forming region is at least 5 times lower than the osmotic pressure in the plug of the second plug type at the plug-forming region where the plug of the second type is formed.

30. (Original) The method of claim 3, where the osmotic pressure in the plug of the first type at the first plug-forming region is at least 10 times lower than the osmotic pressure in the plug of the second plug type at the plug-forming region where the plug of the second type is formed.

31. (Currently Amended) The method of claim 3, where introduction of the first plug-fluids and the second plug-fluids is regulated so that a plug flowing pattern is obtained in a common channel where at least 50% of the plugs of the first plug type are adjacent to the plugs of the second plug type.

32. (Currently Amended) The method of claim 3, where introduction of the first plug-fluids and the second plug-fluids is regulated so that a plug flowing pattern is obtained in a common channel where at least 65% of the plugs of the first plug type are adjacent to the plugs of the second plug type.

33. (Currently Amended) The method of claim 3, where introduction of the first plug-fluids and the second plug-fluids is regulated so that a plug flowing pattern is obtained in a common channel where at least 95% of the plugs of the first plug type are adjacent to the plugs of the second plug type.

34. (Currently Amended) The method of claim 3, where a plug flowing pattern is obtained in which each of the plugs of the first plug type is adjacent to a one plug of the second plug type.

35. (Canceled)

36. (Original) The method of claim 1 or 3, where at least one of the carrier-fluids comprises a fluorinated compound.

37. (Canceled)

38. (Currently Amended) The method of claim 1 or 3, where at least one of the carrier-fluids is permeable to a component in a plug at least one of the plugs.

39. (Original) The method of claim 38, where the component is selected from the group consisting of water, acid, base, buffer and solvent.

40. (Original) The method of claim 1 or 3, where at least one of the carrier-fluids is permeable to water.

41. (Original) The method of claim 3, where the first and second carrier-fluids are permeable to water.

42. (Original) The method of claim 3, where the first and second carrier-fluids are the same.

43. (Currently Amended) The method of claim 3, where the first and second carrier-fluids are ~~the~~ different.

44. (Original) The method of claim 3, where at least one of the carrier-fluids is impermeable to water.

45-48. (Canceled)

49. (Original) The method of claim 1 or 3, where at least one plug-fluid comprises a marker.

50. (Currently Amended) The method of ~~claim 40~~ claim 49, where the marker is an absorption dye or a fluorescent dye.

51. (Currently Amended) The method of claim 1 or 3, further comprising detecting the presence of ~~a crystal in a plug~~ the crystal in at least one of the plugs.

52. (Currently Amended) The method of claim 1 or 3, further comprising analyzing the crystal in ~~the~~ a capillary tube.

53. (Currently Amended) The method of claim 1 or 3, where ~~a crystal the~~ the crystal is removed from a respective channel.

54. (Original) The method of claim 53, where the crystal is collected in a centrifuge tube.

55. (Original) The method of claim 53, where the crystal is collected in a sample tube.

56. (Original) The method of claim 53, where the crystal is collected in a micropipette.

57. (Original) The method of claim 53, where the crystal is collected in a capillary tube.

58. (Original) The method of claim 53, where the crystal is collected in a capillary tube and one or both ends of the capillary tube are sealed.

59. (Original) The method of claim 53, where the crystal is first collected in a capillary tube and then removed from the tube.

60. (Original) The method of claim 1 or 3, where the substrate comprises a material which allows water evaporation from the plugs.

61. (Original) The method of claim 1 or 3, where the substrate is soaked in water prior to crystal formation.

62. (Original) The method of claim 1 or 3, where the substrate is soaked in an aqueous salt solution prior to crystal formation.

63-74. (Canceled)

75. (New) The method of claim 1 or 3, where the solvent of each of the plug-fluids is either the same or different.

76. (New) The method of claim 1, where the plurality of plugs is formed in the first channel when the first plug-fluid, the second plug-fluid, and the third plug-fluid are simultaneously introduced into the plug-forming region of the first channel.

77. (New) The method of claim 1, where the first plug-fluid, the second plug-fluid, and the third plug-fluid are introduced into the plug-forming region of the first channel to form a plurality of drops, the plurality of plugs is formed when at least two of the drops merge in the first channel.